



INVESTOR IN PEOPLE

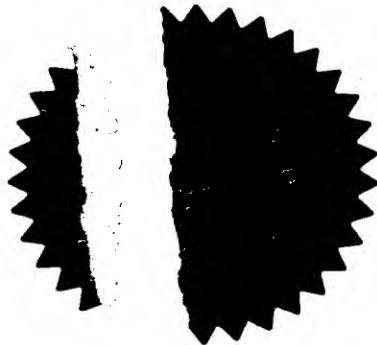
The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



Signed

Dated 15 March 2000

THIS PAGE BLANK (USPTO)



Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

GCM/96148GB

27 MAY 1997

2. Patent application number

(The Patent Office will fill in this part)

97109045

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Allelix Biopharmaceuticals Inc,
6850 Goreway Drive,
Mississauga,
Ontario, Canada L4V 1V7

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

70568400

4. Title of the invention

Neurotrophin Antagonist Compositions

5. Name of your agent (if you have one)

BATCHELLOR, KIRK & CO.

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

2 Pear Tree Court,
Farringdon Road,
London EC1R 0DS

Patents ADP number (if you know it)

315001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

Yes

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body.

See note (d))

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.
Do not count copies of the same document

Continuation sheets of this form

Description 25

Claim(s) 9

Abstract 1

Drawing(s)

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination
(*Patents Form 10/77*)

Any other documents
(please specify)

11.

I/We request the grant of a patent on the basis of this application.

Signature

Batchellor, Kirk & Co.

Date

27th May 1997

12. Name and daytime telephone number of Mr. G. C. March - 0171-253-1563
person to contact in the United Kingdom

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
- b) Write your answers in capital letters using black ink or you may type them.
- c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- e) Once you have filled in the form you must remember to sign and date it.
- f) For details of the fee and ways to pay please contact the Patent Office.

NEUROTROPHIN ANTAGONIST COMPOSITIONS

FIELD OF THE INVENTION

5 The present invention relates to neurotrophin antagonists. In particular, the present invention relates to compositions comprising an effective amount of a compound which inhibits or reduces undesirable neurotrophin activity, and a pharmaceutically acceptable carrier.

BACKGROUND OF THE INVENTION

10 A family of structurally and functionally related neurotrophic factors exist which are collectively known as neurotrophins. The family of neurotrophins includes the nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), 15 neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), neurotrophin-5 (NT-5) and neurotrophin-6 (NT-6).

15 The neurotrophins exhibit similar structural conformations, including three surface β -hairpin loops, a β -strand, an internal reverse turn region, and N- and C-termini. With respect to sequence similarities, the neurotrophins share approximately 50% amino acid identity. The neurotrophins are also functionally similar in that they each exhibit low affinity binding to a receptor known as the "p75 nerve growth factor receptor" or p75^{NGFR}. Each neurotrophin also exhibits binding 20 to a receptor of the tyrosine kinase (trk) family which is of higher affinity than the binding to the p75 receptor. This interaction is believed to be related to neuron survival, but is also involved with neuron differentiation including process formation. 25 The trk receptor-neurotrophin interaction has been found to be more selective than neurotrophin interaction with the p75^{NGFR} receptor. In particular, NGF binds only a trk receptor known as the TrkA receptor, while BDNF, NT-4 and NT-5 exhibit 30 exclusive binding to a TrkB receptor. NT-3 is less selective and, although it binds

primarily with a TrkC receptor, it also exhibits some binding to the TrkA and TrkB receptors (Ibanez et al., EMBO J. 1993, 12:2281).

The neurotrophins function primarily to promote survival of certain classes of
5 peripheral and central neurons both during development and following neuronal damage. NGF, in particular, is involved with the development of neurons in the peripheral nervous system and supports neuronal survival, as well as enhancing and maintaining the differentiated state of neurons. However, in some neurological disease states, the neurotrophins may also support inappropriate neurite outgrowth
10 thereby facilitating the progression of a disease condition. For example, neurotrophins promote the undesirable sprouting of hippocampal "mossy fibres". Such inappropriate sprouting of mossy fibres is a common accompaniment of epilepsy in humans. It is also postulated that the pain experienced by patients suffering from some chronic pain syndromes may be associated with sprouting of
15 sensory pain fibers responsive to NGF in particular into the spinal cord. In other pathological states, such as Alzheimer's disease, aberrant process growth, known as dystrophic neurite formation, is a strong correlate of disease severity.

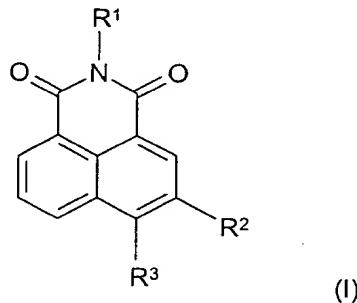
Thus, although the neurotrophins are essential for the normal development
20 and growth of neurons, they may be detrimental under certain circumstances. In such instances, ligands capable of inhibiting or reducing selected neurotrophin-mediated activities would be desirable therapeutically to treat neurodegenerative diseases and conditions including neuropathic pain and to repair nervous system injury.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide compositions capable of inhibiting, or at least reducing, undesirable neurotrophin-mediated activity.

5

In an aspect of the present invention, a composition is provided which comprises a carrier and an effective amount of a compound of Formula I:

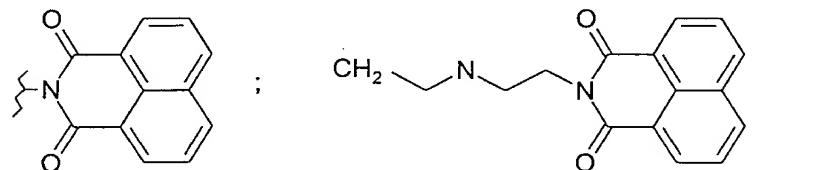


10

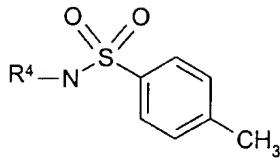
wherein

R¹ is selected from alkyl; aryl-loweralkyl; heterocycle-loweralkyl; amino optionally monosubstituted or disubstituted with a substituent selected from loweralkyl, aryl and hydroxyloweralkyl; benzimidaz-2-yl; phenyl optionally monosubstituted or

15 disubstituted with a substituent selected from amino, loweralkoxy, hydroxy and loweralkyl;



20 and



wherein R⁴ is phenyl optionally monosubstituted or disubstituted with a substituent selected from loweralkyl and halo;

- 5 or R¹ is X(R⁵)(R⁶) wherein X is selected from O and loweralkyl; R⁵ is selected from H and loweralkyl with the proviso that when X is O, R⁵ is H; and R⁶ is selected from carboxy, loweralkanoyl and loweralkoxycarbonyl; and
R² and R³ are independently selected from H, NO₂, halo, di(loweralkyl)amino, cyano, C(O)OH, phenyl-S-, loweralkyl, and Z(O)OR⁷ wherein Z is selected from C
10 and S and R⁷ is selected from H, loweralkylamino and arylamino;
and pharmaceutically acceptable salts and *in vivo* hydrolyzable esters thereof.

In a further aspect of the present invention, there is provided a method for inhibiting a neurotrophin-mediated activity comprising the step of exposing neurons
15 to a composition as described above.

A further aspect of the present invention provides a method for inhibiting neurotrophin-mediated activity in a mammal comprising the step of administering a composition as described above to said mammal.

20

These and other aspects of the present invention will be described in greater detail hereinbelow.

DETAILED DESCRIPTION OF THE INVENTION

The term "alkyl" as used herein means straight and branched chain alkyl radicals containing from one to eight carbon atoms and includes methyl, ethyl, propyl, isopropyl, t-butyl, pentyl, hexyl, heptyl, octyl and the like.

The term "loweralkyl" as used herein means straight and branched chain alkyl radicals containing from one to four carbon atoms and includes methyl, ethyl, propyl, isopropyl, tert-butyl and the like.

10

The term "alkoxy" as used herein means straight and branched chain alkoxy radicals containing from one to eight carbon atoms and includes methoxy, ethoxy, tert-butoxy and the like.

15

The term "loweralkoxy" as used herein means straight and branched chain alkoxy radicals containing from one to four carbon atoms and includes methoxy, ethoxy, tert-butoxy and the like.

20

The term "loweralkanoyl" as used herein means straight and branched chain 1-oxoalkylradicals containing from 1 to 8 carbon atoms.

25

The term "aryl" as used herein means a 5 or 6 membered aromatic ring or heteroaromatic ring containing 1, 2 or 3 heteroatoms independently selected from O, N and S, and includes phenyl, pyridyl, thieryl, furanyl, pyrrolo, imidazolo and the like.

The term "heterocycle" as used herein means a five or six membered, non-aromatic ring optionally containing one or more double bonds and one or two heteroatoms selected from O, S, and N, and includes dihydropyran,

tetrahydropyran, tetrahydrofuryl, azacyclohexane, azacyclohexene, dihydrothiopyran, tetrahydrothiopyran, morpholino and the like).

The term "halo" as used herein means halide and includes fluoro, chloro,
5 bromo and iodo.

As used herein, *in vivo* hydrolyzable esters are those readily hydrolyzable
esters, attached at the hydroxyl group of compounds of Formula I, which are
known and used in the pharmaceutical industry and include α -acyloxyalkyl and
10 esters of C₃₋₂₀-fatty acids.

As it is used herein, the term "neurotrophin" refers to neurotrophic factors
that are structurally homologous to NGF, i.e. include three surface β -hairpin loops,
a β -strand, an internal reverse turn region, and N- and C- termini, and which
15 promote at least one of neuron survival and neuron differentiation, as determined
using assays of conventional design such as the *in vitro* assay exemplified herein
and described by Riopelle *et al.* in Can. J. of Phys. and Pharm., 1982, 60:707.
Mammalian nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF),
neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), neurotrophin-5 (NT-5) and
20 neurotrophin-6 (NT-6) are examples of neurotrophins.

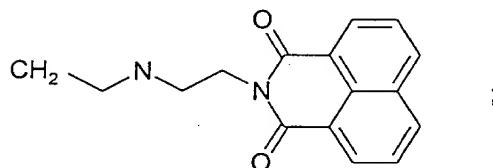
"Neurotrophin-mediated activity" is a biological activity that is normally
promoted, either directly or indirectly, in the presence of a neurotrophin.
Neurotrophin-mediated activities include, for example, neurotrophin binding to the
25 p75^{NGFR} receptor or neurotrophin binding to one of the trk receptors, neuron
survival, neuron differentiation including neuron process formation and neurite
outgrowth, and biochemical changes such as enzyme induction. A biological
activity that is mediated by a particular neurotrophin, e.g. NGF, is referred to herein
by reference to that neurotrophin, e.g. NGF-mediated activity. To determine the
30 ability of a compound to inhibit a neurotrophin-mediated activity, conventional *in*

vitro and *in vivo* assays can be used. For example, a receptor binding assay, such as the assay described herein in Example 1, can be used to assess the extent to which a compound inhibits neurotrophin/receptor binding. Inhibition of neurite survival and outgrowth can be determined using the *in vitro* assay described by
5 Riopelle et al. in the Can. J. of Phys. and Pharm., 1982, 60:707, illustrated herein in Example 2.

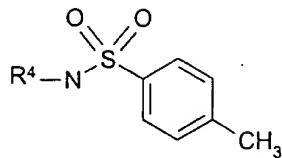
The present invention relates to compositions comprising an effective amount of a compound of Formula I, or pharmaceutically acceptable salts or *in vivo* hydrolyzable esters thereof (hereinafter referred to as a compound of Formula I),
10 which inhibits neurotrophin-mediated activity, and a pharmaceutically acceptable carrier.

In embodiments of the invention, compounds of Formula I include those in
15 which R¹ is selected from alkyl; aryl-loweralkyl; heterocycle-loweralkyl; amino optionally monosubstituted or disubstituted with a substituent selected from loweralkyl and hydroxyloweralkyl; benzimidaz-2-yl; phenyl optionally monosubstituted or disubstituted with a substituent selected from amino, loweralkoxy, hydroxy and loweralkyl;

20



and



wherein R⁴ is phenyl optionally monosubstituted or disubstituted with a substituent selected from loweralkyl and halo;

- 5 or R¹ is X(R⁵)(R⁶) wherein X is selected from O and lower alkyl; R⁵ is selected from H and loweralkyl with the proviso that when X is O, R⁵ is H; and R⁶ is selected from carboxy, loweralkanoyl and loweralkoxycarbonyl; and
- R² and R³ are independently selected from H, NO₂, halo, di(loweralkyl)amino, and phenyl-S-;
- 10 and pharmaceutically acceptable salts and *in vivo* hydrolyzable esters thereof.

In another embodiment of the invention, compounds of Formula I include those in which R¹ is selected from aryl-loweralkyl; heterocycle-loweralkyl; amino optionally monosubstituted or disbstituted with a substituent selected from

- 15 loweralkyl and hydroxyloweralkyl; and benzimidaz-2-yl;
- or R¹ is X(R⁵)(R⁶) wherein X is selected from O and loweralkyl; R⁵ is selected from H and loweralkyl with the proviso that when X is O, R⁵ is H; and R⁶ is selected from carboxy, loweralkanoyl and loweralkoxycarbonyl; and
- R² and R³ are independently selected from H, NO₂, di(loweralkyl)amino, and
- 20 phenyl-S-;
- and pharmaceutically acceptable salts and *in vivo* hydrolyzable esters thereof.

In a further embodiment of the invention, compounds of Formula I include those in which R¹ is amino optionally monosubstituted or disubstituted with a

- 25 substituent selected from loweralkyl and hydroxyloweralkyl;

or R¹ is X(R⁵)(R⁶) wherein X is selected from O and loweralkyl; R⁵ is selected from H and loweralkyl with the proviso that when X is O, R⁵ is H; and R⁶ is selected from carboxy, loweralkanoyl and loweralkoxycarbonyl; and
R² and R³ are independently selected from H and NO₂;
5 and pharmaceutically acceptable salts and *in vivo* hydrolyzable esters thereof.

In a specific embodiment of the invention, compounds of Formula I include:

- 10 N-{5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione}-2-aminoethanol;
N-Dimethylamino-1,3-dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)acetic acid;
N-Acetoxy-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;
15 N-Furfuryl-1,8-naphthalimide;
6-(N,N-Dimethylamino)-2-(benzimidazol-2-yl)naphthalimide;
N-(Pyrid-2-ylethyl)-1,8-naphthalimide;
1,3-Dioxo-6-phenylmercapto-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydro-
benzo[i]isoquinoline;
20 2-{2-(4-methylphenylsulphonamido)phenyl}-6-(N,N-dimethylamino)
naphthalimide;
1,3-Dioxo-2-{2-(4-methylphenylsulphonamido)phenyl}-1,2,3,4-tetrahydro-
benzo[i]isoquinoline;
N-Octyl-5-nitronaphthalimide;
25 5-Bromo-1,3-dioxo-N-methylpyrid-3-yl-1,2,3,4-tetrahydrobenzo-
[i]isoquinoline;
1,3-Dioxo-5-nitro-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydro[i]isoquinoline;
6-Nitro-2-(tetrahydrofuran-2-ylmethyl)naphthalimide;
N-(1,3-Dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;
30 Naphthalicacid-N-aminoimide;

2-{2-(4-Methylbenzsulphonamido)-4,5-dichlorophenyl}naphthalimide;
3-Nitro-1,8-(N-propioncarboxylate)succinamidonaphthalene;
1,3-Dioxo-2-(2-aminophenyl)-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
6-Nitro-2-(pyrid-3-methyl)naphthalimide;
5 3-Amino-7,4-bis(ethyl-1,3-dioxo)-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
2-(Benzimidaz-2-yl)-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
2-(2-Aminophenyl)naphthalimide;
1,3-Dioxo-2-{4,5-dimethyl-2-(4-methylphenylsulphonamido)phenyl}-
1,2,3,4-tetrahydrobenzo[i]isoquinoline;
10 3-Methyl-3-(1,3-dioxo-5-nitro(1H,3H)benz[de]isoquinolyl)butyric acid
methylester;
1,3-Dioxo-N-methyltetrahydrofurfur-2-yl-5-nitro-1,2,3,4-tetrahydro-
[i]isoquinoline;
N-(4-Ethoxyphenyl)-5-nitronaphthalimide;
15 6-Nitro-2-(furfuryl)naphthalimide;
Ethyl-5-nitro-1,3-dioxo-1H-benz[de]isoquinoline-2-3H-acetate;
Naphthalicacid-N,N'-diimide;
2-(2-Hydroxyphenyl)naphthalimide;
5-Amino-N-butylnaphthalimide;
20 1,3-Dioxo-5-nitro-n-propylmorpholino-1,2,3,4-tetrahydrobenzo
[i]isoquinoline;
6-Nitro-2-(pyrid-2-ylethyl)naphthalimide;
N-Methylnaphthalimide;
N-(Pyrid-2-ylmethyl)naphthalimide;
25 N-(3,5-Dimethylphenyl)-1,8-naphthalimide;
6-Bromo-N-dimethylamino-1,3-dioxo-1,2,3,4-tetrahydrobenzo-
[i]isoquinoline;
N-(1,3-Dioxo-6-phenylmercapto-1,2,3,4-tetrahydrobenzo[i]isoquinoline)-
aminoethanol; and
30 N-Anilino-1,8-naphthalimide.

In a preferred embodiment of the invention, compounds of Formula I include:

- N-{5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione}-2-aminoethanol;
- 5 N-Dimethylamino-1,3-dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
- N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)acetic acid;
- N-Acetoxy-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
- N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;
- N-Furyl-1,8-naphthalimide;
- 10 6-(N,N-Dimethylamino)-2-(benzimidazol-2-yl)naphthalimide;
- N-(Pyrid-2-ylethyl)-1,8-naphthalimide;
- 1,3-Dioxo-6-phenylmercapto-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydro-
- benzo[i]isoquinoline;
- 2-{2-(4-methylphenylsulphonamido)phenyl}-6-(N,N-dimethylamino)
- 15 naphthalimide;
- 1,3-Dioxo-2-{2-(4-methylphenylsulphonamido)phenyl}-1,2,3,4-tetrahydro-
- benzo[i]isoquinoline;
- N-Octyl-5-nitronaphthalimide;
- 5-Bromo-1,3-dioxo-N-methylpyrid-3-yl-1,2,3,4-tetrahydrobenzo-
- 20 [i]isoquinoline;
- 1,3-Dioxo-5-nitro-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydro[i]isoquinoline;
- 6-Nitro-2-(tetrahydrofuran-2-ylmethyl)naphthalimide;
- N-(1,3-Dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;
- Naphthalicacid-N-aminoimide;
- 25 2-{2-(4-Methylbenzsulphonamido)-4,5-dichlorophenyl}naphthalimide;
- 3-Nitro-1,8-(N-propioncarboxylate)succinamidonaphthalene;
- 1,3-Dioxo-2-(2-aminophenyl)-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
- 6-Nitro-2-(pyrid-3-methyl)naphthalimide;
- 3-Amino-7,4-bis(ethyl-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline);
- 30 2-(Benzimidaz-2-yl)-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline; and

2-(2-Aminophenyl)naphthalimide.

In a more preferred embodiment of the invention, compounds of Formula I include:

5

N-{5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione}-2-aminoethanol;
N-Dimethylamino-1,3-dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)acetic acid;
N-Acetoxy-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
10 N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;
N-Furfuryl-1,8-naphthalimide;
6-(N,N-Dimethylamino)-2-(benzimidazol-2-yl)naphthalimide;
N-(Pyrid-2-ylethyl)-1,8-naphthalimide; and
1,3-Dioxo-6-phenylmercapto-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydro-
15 benzo[i]isoquinoline.

In a most preferred embodiment of the invention, compounds of Formula I include:

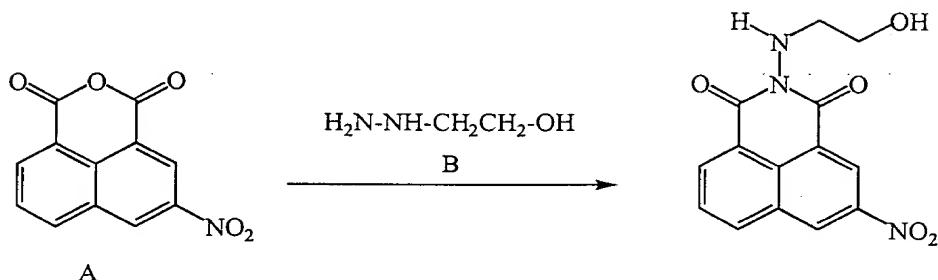
20

N-{5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione}-2-aminoethanol;
N-Dimethylamino-1,3-dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)acetic acid;
N-Acetoxy-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline; and
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol.

25

The compounds of the present invention can be prepared by techniques well known in the art. For example, the compound of formula I wherein R¹ is CH₂CH₂OH, R² is H and R³ is NO₂ can be prepared by coupling naphthalic anhydride reagent A with hydrazino alcohol reagent B in an inert solvent at 30 temperatures in the range of 80-120 °C. The preferred condition for this reaction is

refluxing toluene. Both reagent A and reagent B are commercially available or can be prepared using procedures known to one skilled in the art.



5

Acid addition salts of the compounds of Formula I are most suitably formed from pharmaceutically acceptable acids, and include for example those formed with inorganic acids e.g. hydrochloric, sulphuric or phosphoric acids and organic acids e.g. succinic, maleic, acetic or fumaric acid. Other non-pharmaceutically acceptable salts e.g. oxalates may be used for example in the isolation of the compound of Formula I for laboratory use, or for subsequent conversion to a pharmaceutically acceptable acid addition salt. Also included within the scope of the invention are solvates and hydrates of the invention.

15

The conversion of a given compound salt to a desired compound salt is achieved by applying standard techniques, in which an aqueous solution of the given salt is treated with a solution of base e.g. sodium carbonate or potassium hydroxide, to liberate the free base which is then extracted into an appropriate solvent, such as ether. The free base is then separated from the aqueous portion, 20 dried, and treated with the requisite acid to give the desired salt.

In vivo hydrolyzable esters of the compounds of Formula I can be formed by treating it with the acid chloride of the desired ester in the presence of a base in an

inert solvent such as methylene chloride or chloroform. Suitable bases include triethylamine or pyridine.

The compositions of the present invention are useful to inhibit or reduce
5 undesirable neurotrophin activity both *in vitro* and *in vivo*. Thus, in an aspect of the invention, a composition comprising an effective amount of a compound of Formula I and a suitable carrier is provided. By "suitable carrier" is meant a carrier which admixes with the compound of Formula I to yield a composition suitable for the application for which it is to be used. By "effective amount" is meant an
10 amount of the compound sufficient to inhibit an undesired neurotrophin-mediated activity to a measurable extent, preferably by about 20%, more preferably by about 40%, most preferably by about 50%, as determined using assays of conventional design such as those described herein in the specific examples.

15 The present composition has use as a media supplement to prevent undesirable neurotrophin-mediated activity of neuron cells *in vitro*. For example, primary sensory neurons require NGF for survival in cell culture; however, NGF also influences neuron differentiation, notably process formation and outgrowth, which are undesirable for the use of primary sensory neurons in cell culture. Thus,
20 to preserve neuron survival *in vitro* while inhibiting cell differentiation, NGF is added to the cell culture media along with the compound of Formula I. For addition to the cell culture, the compound is first combined with a carrier which will not adversely affect the growth of the cells in culture. Such carriers will include, for example, physiologically acceptable fluids such as water or any other fluid suitable for
25 addition to the cell culture. Alternatively, the compound can be combined with media suitable for culturing neuronal cells prior to being added to the cell culture. To be effective to prevent neuron differentiation, the concentration of the compound in the cell culture will be in the range of from about 1-500 µM, and preferably from about 1-100 µM. The optimal concentration of compound for use

in preventing neuron differentiation in cell culture will, of course, vary depending on the extent of inhibition desired as well as the type of neuronal cells involved.

Compositions for *in vivo* administration, e.g. for treating neurological

5 conditions such as epilepsy or Alzheimer's disease, or for treating chronic pain, are also contemplated. Such compositions comprise a therapeutically effective amount of the compound of Formula I together with a pharmaceutically acceptable carrier. In this context, the term "pharmaceutically acceptable" means acceptable for use in the pharmaceutical and veterinary arts, i.e. non-toxic and not adversely 10 affecting the activity of the compound. The term "therapeutically effective amount" means an amount of the compound sufficient to reduce undesirable neurotrophin-mediated activity, as determined using assays of conventional design, in an inflicted individual without causing adverse effects.

15 Pharmaceutically acceptable carriers useful to prepare compositions for *in vivo* administration include conventional carriers used in formulating drugs, such as diluents, excipients and the like. Reference may be made to "Remington's Pharmaceutical Sciences", 17th Ed., Mack Publishing Company, Easton, Penn., 1985, for guidance on drug formulations generally. As will be appreciated, the 20 pharmaceutical carriers used to prepare compositions in accordance with the present invention will depend on the dosage form to be used to treat the inflicted individual.

According to one embodiment of the invention, a compound of Formula I is 25 formulated for administration by injection intraventricularly, and is accordingly provided as an aqueous solution in sterile and pyrogen-free form and optionally buffered or made isotonic. Thus, the compound may be administered in distilled water or, more desirably, in saline or 5% dextrose solution. Water solubility of the 30 compound of the invention may be enhanced, if desired, by incorporating into the composition a solubility enhancer, such as acetyltrimethylammonium bromide or

chloride. Lyoprotectants, such as mannitol, sucrose or lactose and buffer systems, such as acetate, citrate and phosphate may also be included in the formulation, as may bulking agents such as serum albumin.

- 5 For use in treating individuals with a neurological condition, precise dosage sizes of a pharmaceutical composition appropriate for treatment are established in appropriately controlled trials, and will correspond to an amount of a compound of Formula I that reduces undesirable neurotrophin-mediated activity without causing intolerable side effects to the individual being treated. It is anticipated that an
10 effective treatment regimen for patients will involve the intraventricular administration of dosages which achieve a level of the compound in the spinal fluid of the individual being treated of about 1-500 µM. It will be appreciated, of course, that the dosage sizes required to attain this *in vivo* concentration will vary according to the route of administration, the frequency of administration, on the
15 individual being treated and on the neurological condition being treated.

Specific embodiments of the present invention are described in more detail in the following examples which are not to be construed as limiting.

- 20 Example 1 - Binding of [¹²⁵I]NGF to PC12 cells in the presence and absence of BDNF

The ability of the compounds of Formula I to antagonize NGF interaction
25 with the p75 and trkA receptors was determined as follows.

(A) Iodination of NGF

NGF was labelled using the Lactoperoxidase labelling method (Sutter *et al.*, *J.
30 Biol. Chem.*, 1979) and the labelled NGF was separated from radiolabelling

agents and free iodide using a PD-10 Sephadex G-25 column.

(B) Cell culture and cell preparation

PC12 cells were grown in RPMI with 10% heat inactivated donor horse serum
5 and 5% fetal calf serum. Cells were harvested for binding by washing off the media with calcium-magnesium free balanced salt solution (Gey's solution) and incubated in 5 ml Gey's solution at 37°C for 15 minutes. Cells were pelleted by centrifugation and suspended in Hepes-Krebs Ringer buffer (HKR) (10 mM Hepes pH7.35, containing 125 mM NaCl, 4.8 mM KCl, 1.3 mM CaCl₂, 1.2 mM 10 MgSO₄, 1.2 mM KH₂PO₄, 1 mg/ml BSA and 1.0 mg/ml glucose) at a concentration of 4x10⁶/ml and kept on ice.

(C) NGF Binding

15 The reaction was performed in a 96 well plate. Suspended cells (150ul, 10⁶ cells) were added to ¹²⁵I-NGF (final concentration of 0.5 nM) and the competing compound of Formula I in a final volume of 300 ul of HKR buffer. The plates were incubated with shaking for 2 hr at 4°C. At the end of the incubation, 100ul aliquots of the reaction sample were added to 400ul microcentrifuge tubes
20 containing 200 ul of 10% glycerol in HKR buffer. The tubes were centrifuged for 1 minute at ~5000rpm and the tip containing the cell pellet was cut off. Radioactivity bound to the cells was determined by measuring the ¹²⁵I-NGF associated with each pellet in a gamma counter. Specific binding is calculated as the difference between the amount of ¹²⁵I-NGF bound in the absence (total) and
25 presence (NSB) of 50nM unlabeled NGF. TrkA binding is determined similarly except 10nM BDNF is added to all reactions. Table 1 summarizes the values obtained from this experiment for the inhibition of binding of NGF to P75 and TrkA by compounds of Formula I.

TABLE 1

Compound	% Inhibition at 100 uM
N-Dimethylamino-1,3-dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline	59
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)acetic acid	58
N-Acetoxy-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline	57
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)-aminoethanol	44
N-Furfuryl-1,8-naphthalimide	36
6-(N,N-Dimethylamino)-2-(benzimidazol-2-yl)naphthalimide	36
N-(Pyrid-2-ylethyl)-1,8-naphthalimide	35
1,3-Dioxo-6-phenylmercapto-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydrobenzo[i]isoquinoline	33
2-{2-(4-methylphenylsulphonamido)phenyl}-6-(N,N-dimethylamino)-naphthalimide	28
1,3-Dioxo-2-{2-(4-methylphenylsulphonamido)phenyl}-1,2,3,4-tetrahydrobenzo[i]isoquinoline	29
N-Octyl-5-nitronaphthalimide	29
5-Bromo-1,3-dioxo-N-methylpyrid-3-yl-1,2,3,4-tetrahydrobenzo[i]-isoquinoline	28
1,3-Dioxo-5-nitro-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydro[i]isoquinoline	28
6-Nitro-2-(tetrahydrofuran-2-ylmethyl)naphthalimide	28
N-(1,3-Dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol	27
Naphthalicacid-N-aminoimide	27
2-[2-(4-Methylbenzsulphonamido)-4,5-dichlorophenyl]naphthalimide	25
3-Nitro-1,8-(N-propioncarboxylate)succinamidonaphthalene	25
1,3-Dioxo-2-(2-aminophenyl)-1,2,3,4-tetrahydrobenzo[i]isoquinoline	24
6-Nitro-2-(pyrid-3-methyl)naphthalimide	23

TABLE 1 (Cont'd)

Compound	% Inhibition at 100 uM
3-Amino-7,4-bis(ethyl-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline	23
2-(Benzimidaz-2-yl)-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline	23
2-(2-Aminophenyl)naphthalimide	20
1,3-Dioxo-2-[4,5-dimethyl-2-(4-methylphenylsulphonamido)phenyl]-1,2,3,4-tetrahydrobenzo[i]isoquinoline	19
N-(4-Ethoxyphenyl)-5-nitronaphthalimide	18
1,3-Dioxo-N-methyltetrahydrofurfur-2-yl-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline	18
3-Methyl-3-(1,3-dioxo-5-nitro(1H,3H)benz[de]isoquinolyl)butyric acid methylester	17
6-Nitro-2-(furfuryl)naphthalimide	17
Ethyl-5-nitro-1,3-dioxo-1H-benz[de]isoquinoline-2-3H-acetate	16
Naphthalicacid-N,N'-diimide	13
2-(2-Hydroxyphenyl)naphthalimide	13
5-Amino-N-butylnaphthalimide	13
1,3-Dioxo-5-nitro-n-propylmorpholino-1,2,3,4-tetrahydrobenzo[i]isoquinoline	11
6-Nitro-2-(pyrid-2-ylethyl)naphthalimide	11
N-Methylnaphthalimide	11
N-(Pyrid-2-ylmethyl)naphthalimide	10
N-(3,5-Dimethylphenyl)-1,8-naphthalimide	9
6-Bromo-N-dimethylamino-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline	5
N-(1,3-Dioxo-6-phenylmercapto-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol	3
N-Anilino-1,8-naphthalimide	1

Example 2 - Inhibition of Neurite Outgrowth

The ability of N-{5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione}-2-aminoethanol (Compound A) to inhibit neurite outgrowth was determined using the
5 following assay.

Eight-day chick embryo dorsal root ganglia (DRG) were freed of meninges and removed aseptically. The DRG were kept at 4 °C at all times. Ganglia from six embryos (40-50 per embryo) were washed in Ca²⁺- and Mg²⁺- free Gey's balanced
10 salt solution (Gibco) and exposed to 0.01% trypsin (Worthington) in the same solution for 10 min at 37 °C. A half-volume of phosphate-buffered Gey's balanced salt solution was added for a further 5 min at 37 °C and the reaction was then terminated with one-third volume of Ham's F12 medium (Gibco) containing 5% fetal calf serum (FCS, Gibco). The ganglia were then triturated using a 5 mL
15 narrow-tip pipette to a single cell suspension. Following filtration through 37-mm nylon mesh (Small Parts Inc., Miami, FL) in a Millipore chamber to remove clumps, the cell suspension was washed through a 500-mL FCS undercut (700 x g for 5 min at 4 °C) and resuspended in 4 mL of Ham's F12 medium plus 5% FCS. The cell suspension was then preplated on a 100-mm Flacon culture dish and incubated for
20 45-60 min at 37 °C in a 5% CO₂ humidified atmosphere. Cells enriched in neurons were decanted for the bioassay, since non-neuronal cells of DRG preferentially stick to the culture substrate.

The inside wells of 96-well Falcon microculture plates were coated with
25 polylysine (0.1 mg/mL) (Sigma) for 4 h at 37 °C (the outside wells were filled with distilled water to provide humidity) and, following a rinse with tissue culture media, 100 mL of neuron-rich cell suspension was added to each well at 10⁵ cells/mL. Ninety (90) mL of NGF solution (prepared in tissue culture media) was then added to each well to a final concentration of 0.25 ng/mL NGF per well. Ten (10) mL of
30 test compound solution, i.e. tissue culture media admixed with a compound of

Formula I, was then added to test wells in duplicate to yield wells containing compound concentrations ranging from 0 μ M - 100 μ M. For control assays, 10 mL of Ham's F12 medium was added to duplicate NGF-containing wells. The plates were covered and incubated in the dark for 24-30 hrs. at 37 °C in a 5% CO₂ humidified atmosphere.

The bioassays were read using a Leitz Diavert microscope with phase optics. To afford adequate optics, the meniscus effect of each well was removed by filling the well with a balanced salt solution until a flat, air-filled interface was achieved at the top of the well. At least 100 neurons per well were counted, and the assay was scored as the ratio of cells bearing neurites greater than one cell diameter to total viable (phase bright) cells. These results are summarized in Table 2.

15

TABLE 2

Group	Ratio	Std Dev	SEM
NGF Control	1.000	0.16	0.06
5 μ M Compound A	1.1	0.42	0.15
50 μ M Compound A	0.16	0.15	0.05
100 μ M Compound A	0.016	0.02	0.01

Example 3 - Animal Models of Neuropathic Pain

For pain related to nerve injury, the compound N-{5-nitro-1H-benz[de]iso-quinoline-1,3(2H)-dione}-2-aminoethanol (Compound A) was tested in nerve-

5 ligated rats for activity against tactile allodynia, thermal hyperalgesia and in direct production of thermal antinociception. The nerve-ligation model is commonly accepted as representing aspects of neuropathic pain reported by humans. Sham operated rats served as appropriate controls for the neuropathic experiments.

10 Nerve Ligation Injury:

Nerve ligation injury was performed according to the method described by Kim and Chung (Pain 50 : 355-363, 1992). Rats were anesthetized with halothane and the vertebrae over the L4 to S2 region were exposed. L5 and L6 15 spinal nerves were exposed, carefully isolated, tightly ligated with 4-0 silk suture distal to the DRG. After ensuring homeostatic stability, the wounds were sutured, and the animals were allowed to recover in the cages. Sham-operated rats were prepared in an identical fashion except that the L5/L6 nerve roots are not ligated.

20 Intrathecal Catheter Placement:

The test compounds were injected through indwelling i. th. catheters. While under anesthesia, PE-10 tubing (8cm) was inserted through an incision made in the atlanto-occipital membrane to the level of the lumbar enlargement of 25 the rat and secured. Drug injections were made in a volume of 5 μ l of 50% aqueous DMSO followed by a 9 μ l saline flush.

Endpoints:

(A) Evaluation of Tactile Allodynia:

5 Mechanical allodynia was determined in the manner described by
Chaplan et al. (J. Neurosci. Meth. 53:55-63, 1994). The paw withdrawal
threshold was determined in response to probing with calibrated von Frey
filaments. The rats were kept in suspended cages with mesh floors and the von
Frey filaments were applied perpendicularly to the plantar surface of the paw of
10 the rat until it buckled slightly, and held for 3-6 sec. A positive response was
indicated by a sharp withdrawal of the paw. The 50% paw withdrawal threshold
was determined by the non-parametric method of Dixon (Ann. rev. Pharmacol.
Toxicol. 20: 441-462, 1980).

15 (B) Evaluation of Thermal Hyperalgesia:

Thermal hyperalgesia was determined by focusing a radiant heat source
onto the plantar surface of the affected paw of nerve-injured or sham-operated
rats. Paw withdrawal latencies are determined by a photodetection device which
20 halts the stimulus and the timer after a maximum cut-off of 40 sec to prevent
tissue damage. The withdrawal latency of sham-operated rats were compared to
those of ligated rats to measure the degree of hyperalgesia.

(C) Evaluation of Acute Nociceptive Responses:

25 Acute nociception was determined by using the nociceptive warm water
tail-flick reflex. This test was performed by placing the tail of the nerve-injured or
sham-operated rats in a heated water bath maintained at 55°C. The latency until
tail withdrawal (rapid flick) from the bath was determined and compared among
30 treatments. A 15 second cut-off was employed to avoid tissue damage.

Compound A and morphine were tested in the nerve-ligated injury model of neuropathic pain using 3 routes of administration: intraperitoneally (i.p.), intrathecally (i.th.) and intracerebroventrically (i.c.v.). The compounds were evaluated for three endpoints: tactile allodynia, thermal hyperalgesia and acute nociception. Compound A is not active when given i.c.v. The results for i.p and i.th. administration are shown in Table 3.

TABLE 3

Summary of the A_{50} doses with 95% Confidence Limits (C.L.) for Compound A and Morphine in L5/L6 Nerve Ligated and Sham-Operated Rats in Models of Tactile Allodynia, Acute Nociception and Thermal Hyperalgesia			
Treatment		Compound A A_{50} (95% C.L.)	Morphine A_{50} (95% C.L.)
Allodynia- Ligated	<i>i.th</i>	34.6 μ g	>100 μ g
	<i>i.p.</i>	38 mg/kg	7.1 mg/kg
Tail Flick- Ligated	<i>i.th</i>	78 μ g	2.3 μ g
	<i>i.p.</i>	27.8 mg/kg	na*
Tail Flick- Sham	<i>i.th</i>	34 μ g	1.45 μ g
	<i>i.p.</i>	25.7 mg/kg	na
Hot Plate- Ligated	<i>i.th</i>	14.8 μ g	na
	<i>i.p.</i>	18.6 mg/kg	na
Hot Plate- Sham	<i>i.th</i>	48.1 μ g	na
	<i>i.p.</i>	34.9 mg/kg	na

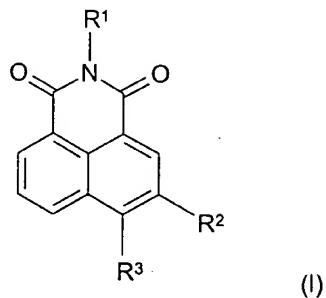
10 * na = not available

Example 4 - Preparation of N-{5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione}-2-aminoethanol (Compound A)

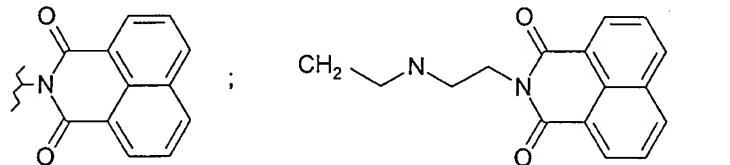
- 3-Nitro-1,8-naphthalic anhydride (1 eq) and 2-hydroxyethylhydrazine (1 eq)
5 are dissolved in toluene and heated to reflux. The reaction is monitored by tlc and halted when all of the starting materials are consumed. The solvent is removed under reduced pressure and the product purified, if necessary, by recrystallization or silica gel chromatography.
- 10 Other compounds of Formula I can be prepared in an analogous manner, or are available commercially from Ryan Scientific Inc., Isle of Palms, South Carolina, U.S.

WE CLAIM:

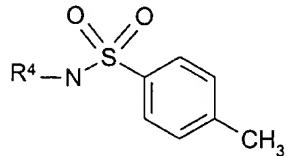
1. A pharmaceutical composition comprising a compound of Formula I,



wherein R¹ is selected from alkyl; aryl-loweralkyl; heterocycle-loweralkyl; amino optionally monosubstituted or disubstituted with a substituent selected from loweralkyl, aryl and hydroxyloweralkyl; benzimidaz-2-yl; phenyl optionally monosubstituted or disubstituted with a substituent selected from amino, loweralkoxy, hydroxy and loweralkyl;

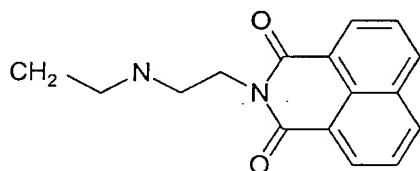


and

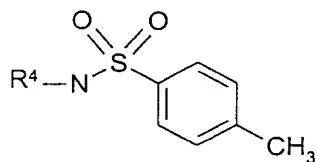


wherein R⁴ is phenyl optionally monosubstituted or disubstituted with a substituent selected from lower alkyl and halo; or R¹ is X(R⁵)(R⁶) wherein X is selected O and loweralkyl; R⁵ is selected from H and loweralkyl with the proviso that when X is O, R⁵ is H; and R⁶ is selected from carboxy, loweralkanoyl and loweralkoxycarbonyl; and R² and R³ are independently selected from H, NO₂, halo, di(loweralkyl)amino, cyano, C(O)OH, phenyl-S-, loweralkyl, and Z(O)OR⁷ wherein Z is selected from C and S and R⁷ is selected from H, loweralkylamino and arylamino; or pharmaceutically acceptable salts or *in vivo* hydrolyzable esters thereof, in an amount effective to inhibit neurotrophin-mediated activity, and a suitable carrier.

2. A pharmaceutical composition according to claim 1, wherein R¹ is selected from alkyl; aryl-loweralkyl; heterocycle-loweralkyl; amino optionally monosubstituted or disbsstituted with a substituent selected from loweralkyl and hydroxyloweralkyl; benzimidaz-2-yl; phenyl optionally monosubstituted or disubstituted with a substituent selected from amino, loweralkoxy, hydroxy and loweralkyl;



and



wherein R⁴ is phenyl optionally monosubstituted or disubstituted with a substituent selected from loweralkyl and halo; or R¹ is X(R⁵)(R⁶) wherein X is selected from O and loweralkyl; R⁵ is selected from H and loweralkyl with the proviso that when X is O, R⁵ is H; and R⁶ is selected from carboxy, loweralkanoyl and loweralkoxy-carbonyl; and R² and R³ are independently selected from H, NO₂, halo, di(loweralkyl)amino, and phenyl-S-.

3. A pharmaceutical composition according to claim 2, wherein R¹ is selected from aryl-loweralkyl; heterocycle-loweralkyl; amino optionally monosubstituted or disbsitutued with a substituent selected from loweralkyl and hydroxyloweralkyl; and benzimidaz-2-yl; or R¹ is X(R⁵)(R⁶) wherein X is selected from O and loweralkyl; R⁵ is selected from H and loweralkyl with the proviso that when X is O, R⁵ is H; and R⁶ is selected from carboxy, loweralkanoyl and loweralkoxycarbonyl; and R² and R³ are independently selected from H, NO₂, di(loweralkyl)amino, and phenyl-S-.

4. A pharmaceutical composition according to claim 3, wherein R¹ is amino optionally monosubstituted or disbstitutued with a substituent selected from loweralkyl and hydroxyloweralkyl; or R¹ is X(R⁵)(R⁶) wherein X is selected from O and loweralkyl; R⁵ is selected from H and loweralkyl with the proviso that when X is O, R⁵ is H; and R⁶ is selected from carboxy, loweralkanoyl and loweralkoxy-carbonyl; and R² and R³ are independently selected from H and NO₂.

5. A pharmaceutical composition according to claim 1 wherein the compound of Formula I is selected from the group consisting of:

N-{5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione}-2-aminoethanol;
N-Dimethylamino-1,3-dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)acetic acid;
N-Acetoxy-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;

N-Furfuryl-1,8-naphthalimide;
6-(N,N-Dimethylamino)-2-(benzimidazol-2-yl)naphthalimide;
N-(Pyrid-2-ylethyl)-1,8-naphthalimide;
1,3-Dioxo-6-phenylmercapto-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydro-
benzo[i]isoquinoline;
2-{2-(4-methylphenylsulphonamido)phenyl}-6-(N,N-dimethylamino)-
naphthalimide;
1,3-Dioxo-2-{2-(4-methylphenylsulphonamido)phenyl}-1,2,3,4-tetrahydro-
benzo[i]isoquinoline;
N-Octyl-5-nitronaphthalimide;
5-Bromo-1,3-dioxo-N-methylpyrid-3-yl-1,2,3,4-tetrahydrobenzo-
[i]isoquinoline;
1,3-Dioxo-5-nitro-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydro[i]isoquinoline;
6-Nitro-2-(tetrahydrofuran-2-ylmethyl)naphthalimide;
N-(1,3-Dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;
Naphthalicacid-N-aminoimide;
2-{2-(4-Methylbenzsulphonamido)-4,5-dichlorophenyl}naphthalimide;
3-Nitro-1,8-(N-propioncarboxylate)succinamidonaphthalene;
1,3-Dioxo-2-(2-aminophenyl)-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
6-Nitro-2-(pyrid-3-methyl)naphthalimide;
3-Amino-7,4-bis(ethyl-1,3-dioxo)-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
2-(Benzimidaz-2-yl)-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
2-(2-Aminophenyl)naphthalimide;
1,3-Dioxo-2-{4,5-dimethyl-2-(4-methylphenylsulphonamido)phenyl}-
1,2,3,4-tetrahydrobenzo[i]isoquinoline;
3-Methyl-3-(1,3-dioxo-5-nitro(1H,3H)benz[de]isoquinolyl)butyric acid
methylester;
1,3-Dioxo-N-methyltetrahydrofurfur-2-yl-5-nitro-1,2,3,4-tetrahydro-
[i]isoquinoline;
N-(4-Ethoxyphenyl)-5-nitronaphthalimide;

6-Nitro-2-(furfuryl)naphthalimide;
Ethyl-5-nitro-1,3-dioxo-1H-benz[de]isoquinoline-2-3H-acetate;
Naphthalic acid-N,N'-diimide;
2-(2-Hydroxyphenyl)naphthalimide;
5-Amino-N-butyl naphthalimide;
1,3-Dioxo-5-nitro-n-propylmorpholino-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
6-Nitro-2-(pyrid-2-ylethyl)naphthalimide;
N-Methylnaphthalimide;
N-(Pyrid-2-ylmethyl)naphthalimide;
N-(3,5-Dimethylphenyl)-1,8-naphthalimide;
6-Bromo-N-dimethylamino-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
N-(1,3-Dioxo-6-phenylmercapto-1,2,3,4-tetrahydrobenzo[i]isoquinoline)-aminoethanol; and
N-Anilino-1,8-naphthalimide.

6. A pharmaceutical composition according to claim 2 wherein the compound of Formula I is selected from the group consisting of:

N-{5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione}-2-aminoethanol;
N-Dimethylamino-1,3-dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)acetic acid;
N-Acetoxy-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;
N-Furfuryl-1,8-naphthalimide;
6-(N,N-Dimethylamino)-2-(benzimidazol-2-yl)naphthalimide;
N-(Pyrid-2-ylethyl)-1,8-naphthalimide;
1,3-Dioxo-6-phenylmercapto-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydrobenzo[i]isoquinoline;

2-{2-(4-methylphenylsulphonamido)phenyl}-6-(N,N-dimethylamino)-naphthalimide;

1,3-Dioxo-2-{2-(4-methylphenylsulphonamido)phenyl}-1,2,3,4-tetrahydrobenzo[i]isoquinoline;

N-Octyl-5-nitronaphthalimide;

5-Bromo-1,3-dioxo-N-methylpyrid-3-yl-1,2,3,4-tetrahydrobenzo[i]isoquinoline;

1,3-Dioxo-5-nitro-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydro[i]isoquinoline;

6-Nitro-2-(tetrahydrofuran-2-ylmethyl)naphthalimide;

N-(1,3-Dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;

Naphthalicacid-N-aminoimide;

2-{2-(4-Methylbenzsulphonamido)-4,5-dichlorophenyl}naphthalimide;

3-Nitro-1,8-(N-propioncarboxylate)succinamidonaphthalene;

1,3-Dioxo-2-(2-aminophenyl)-1,2,3,4-tetrahydrobenzo[i]isoquinoline;

6-Nitro-2-(pyrid-3-methyl)naphthalimide;

3-Amino-7,4-bis(ethyl-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline);

2-(Benzimidaz-2-yl)-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline; and

2-(2-Aminophenyl)naphthalimide.

7. A pharmaceutical composition according to claim 3 wherein the compound of Formula I is selected from the group consisting of:

N-{5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione}-2-aminoethanol;

N-Dimethylamino-1,3-dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline;

N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)acetic acid;

N-Acetoxy-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline;

N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;

N-Furfuryl-1,8-naphthalimide;

6-(N,N-Dimethylamino)-2-(benzimidazol-2-yl)naphthalimide;

N-(Pyrid-2-ylethyl)-1,8-naphthalimide; and

1,3-Dioxo-6-phenylmercapto-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydrobenzo[i]isoquinoline.

8. A pharmaceutical composition according to claim 4 wherein the compound of Formula I is selected from the group consisting of:

N-{5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione}-2-aminoethanol;
N-Dimethylamino-1,3-dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)acetic acid;
N-Acetoxy-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline; and
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol.

9. A pharmaceutical composition as defined in claim 1, which inhibits NGF-mediated activity.

10. A method for inhibiting a neurotrophin-mediated activity comprising the step of exposing neuron cells to an effective amount of a composition as defined in claim 1.

11. A method for inhibiting a neurotrophin-mediated activity in a mammal comprising the step of administering to said mammal a therapeutically effective amount of a composition as defined in claim 1.

12. A method as defined in claim 11, wherein said composition is administered intraventricularly.

13. A hydrolyzable ester of a compound selected from the group consisting of:

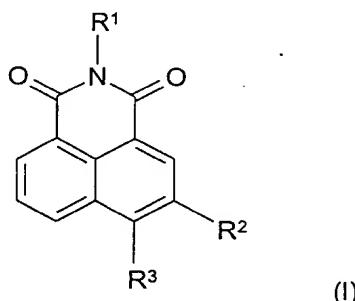
N-{5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione}-2-aminoethanol;
N-Dimethylamino-1,3-dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)acetic acid;
N-Acetoxy-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline;

N-(1,3-Dioxo-5-nitro-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;
N-Furfuryl-1,8-naphthalimide;
6-(N,N-Dimethylamino)-2-(benzimidazol-2-yl)naphthalimide;
N-(Pyrid-2-ylethyl)-1,8-naphthalimide;
1,3-Dioxo-6-phenylmercapto-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydro-
benzo[i]isoquinoline;
2-{2-(4-methylphenylsulphonamido)phenyl}-6-(N,N-dimethylamino)-
naphthalimide;
1,3-Dioxo-2-{2-(4-methylphenylsulphonamido)phenyl}-1,2,3,4-tetrahydro-
benzo[i]isoquinoline;
N-Octyl-5-nitronaphthalimide;
5-Bromo-1,3-dioxo-N-methylpyrid-3-yl-1,2,3,4-tetrahydrobenzo-
[i]isoquinoline;
1,3-Dioxo-5-nitro-N-(pyrid-2-ylethyl)-1,2,3,4-tetrahydro[i]isoquinoline;
6-Nitro-2-(tetrahydrofuran-2-ylmethyl)naphthalimide;
N-(1,3-Dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline)aminoethanol;
Naphthalicacid-N-aminoimide;
2-{2-(4-Methylbenzsulphonamido)-4,5-dichlorophenyl}naphthalimide;
3-Nitro-1,8-(N-propioncarboxylate)succinamidonaphthalene;
1,3-Dioxo-2-(2-aminophenyl)-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
6-Nitro-2-(pyrid-3-methyl)naphthalimide;
3-Amino-7,4-bis(ethyl-1,3-dioxo)-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
2-(Benzimidaz-2-yl)-1,3-dioxo-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
2-(2-Aminophenyl)naphthalimide;
1,3-Dioxo-2-{4,5-dimethyl-2-(4-methylphenylsulphonamido)phenyl}-
1,2,3,4-tetrahydrobenzo[i]isoquinoline;
3-Methyl-3-(1,3-dioxo-5-nitro(1H,3H)benz[de]isoquinolyl)butyric acid
methylester;
1,3-Dioxo-N-methyltetrahydrofurfur-2-yl-5-nitro-1,2,3,4-tetrahydro-
[i]isoquinoline;

N-(4-Ethoxyphenyl)-5-nitronaphthalimide;
6-Nitro-2-(furfuryl)naphthalimide;
Ethyl-5-nitro-1,3-dioxo-1H-benz[de]isoquinoline-2-3H-acetate;
Naphthalicacid-N,N'-diimide;
2-(2-Hydroxyphenyl)naphthalimide;
5-Amino-N-butylnaphthalimide;
1,3-Dioxo-5-nitro-n-propylmorpholino-1,2,3,4-tetrahydrobenzo[i]isoquinoline;
6-Nitro-2-(pyrid-2-ylethyl)naphthalimide;
N-Methylnaphthalimide;
N-(Pyrid-2-ylmethyl)naphthalimide;
N-(3,5-Dimethylphenyl)-1,8-naphthalimide;
6-Bromo-N-dimethylamino-1,3-dioxo-1,2,3,4-tetrahydrobenzo-[i]isoquinoline;
N-(1,3-Dioxo-6-phenylmercapto-1,2,3,4-tetrahydrobenzo[i]isoquinoline)-
aminoethanol; and
N-Anilino-1,8-naphthalimide.

ABSTRACT

A pharmaceutical composition comprising a compound of Formula I



wherein R¹ is selected from, *inter alia*, alkyl, aryl-loweralkyl, heterocycle-loweralkyl, optionally substituted amino; benzimidaz-2-yl, and optionally substituted phenyl; or R¹ is X(R⁵)(R⁶) wherein X is selected O and lower alkyl; R⁵ is selected from H and loweralkyl with the proviso that when X is O, R⁵ is H; and R⁶ is selected from carboxy, loweralkanoyl and loweralkoxycarbonyl; and R² and R³ are independently selected from H, NO₂, halo, di(loweralkyl)amino, cyano, C(O)OH, phenyl-S-, loweralkyl, and Z(O)OR⁷ wherein Z is selected from C and S and R⁷ is selected from H, loweralkylamino and arylamino; or pharmaceutically acceptable salts or *in vivo* hydrolyzable esters thereof, in an amount effective to inhibit neurotrophin-mediated activity, and a suitable carrier, is described.

The compositions are useful to inhibit undesirable neurotrophin-mediated activity such as the neurite outgrowth that occurs in some neurodegenerative disease states.

THIS PAGE BLANK (USPTO)